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14. ABSTRACT
Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.
Tuberous sclerosis complex (TSC) is an autosomal genetic disorder which affects about 1 in 6,000 newborns. The disease is caused by inactivating mutations in either of two related tumor suppressor genes, TSC1 (encoding hamartin) or TSC2 (encoding tuberin). The TSC proteins regulate the mTOR pathway and are critical in cell growth and proliferation. TSC gene carriers are born with one defective copy and if they lose the normal copy is somatic tissues, pathologic lesions develop which can affect multiple organs in the body. Focal pathologic lesions in the brain, including cortical tubers and subependymal nodules, are seen in the majority (>90%) of TSC patients, and disrupt neuronal architecture causing epilepsy and obstruction of ventricles, respectively (Short et al., 1995). In a magnetic resonance imaging (MRI) study about one third of subependymal nodules were observed to grow over a 4-year period postnatally (Katz et al., 2012) with the potential to block cerebrospinal fluid (CSF) flow leading to fatal hydrocephalus. Early surgical removal of subependymal nodules has been recommended, but has neurosurgical risks (Berhouma, 2010).

15. SUBJECT TERMS-

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

   Tuberous sclerosis complex (TSC) is an autosomal genetic disorder which affects about 1 in 6,000 newborns. The disease is caused by inactivating mutations in either of two related tumor suppressor genes, *TSC1* (encoding hamartin) or *TSC2* (encoding tuberin). The TSC proteins regulate the mTOR pathway and are critical in cell growth and proliferation. TSC gene carriers are born with one defective copy and if they lose the normal copy is somatic tissues, pathologic lesions develop which can affect multiple organs in the body. Focal pathologic lesions in the brain, including cortical tubers and subependymal nodules, are seen in the majority (>90%) of TSC patients, and disrupt neuronal architecture causing epilepsy and obstruction of ventricles, respectively (Short et al., 1995). In a magnetic resonance imaging (MRI) study about one third of subependymal nodules were observed to grow over a 4-year period postnatally (Katz et al., 2012) with the potential to block cerebrospinal fluid (CSF) flow leading to fatal hydrocephalus. Early surgical removal of subependymal nodules has been recommended, but has neurosurgical risks (Berhouma, 2010).

   A number of models of TSC brain lesions have been described in conditional knock-out Tsc1 mice, which include cortical defects, tuber-like structures and subependymal nodules (e.g. Feliciano et al., 2011). Mice typically die early of unknown causes, one of which appears to be hydrocephalus due to restriction of CSF flow. Recent advances in gene therapy have provided a safe means of gene replacement therapy in human clinical trials for neurologic diseases using adeno-associated virus (AAV) vectors (including serotypes 1 & 2, AAV1 and AAV2) which can be injected directly into the brain parenchyma or ventricles or, in the case of serotype 9 (and other serotypes such as rh8; Yang et al., 2014) into the circulation with transduction of brain threatening subependymal nodules may be induced to regress by injection of an enzyme replacement vector, such as AAV encoding hamartin in the case of Tsc1, instead of undertaking more invasive neurosurgery.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

   Tuberous sclerosis complex, TSC, TSC1, TSC2, Gene therapy, AAV, neurons.

3. **OVERALL PROJECT SUMMARY:** Summarize the progress during appropriate reporting period (single annual or comprehensive final). This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW in a summary of Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion. Key methodology used during the reporting period, including a description of any changes to originally proposed methods, shall be summarized. Data supporting research conclusions, in the form of figures and/or tables, shall be embedded in the text, appended, or referenced to appended manuscripts. Actual or anticipated problems or delays and actions or plans to resolve them shall be included. Additionally, any changes in approach and reasons for these changes shall be reported. **Any change that is substantially different from the original approved SOW (e.g., new or modified tasks, objectives, experiments, etc.) requires review by the Grants Officer's Representative and final approval by USAMRAA Grants Officer through an award modification prior to initiating any changes.**

Our studies were designed to evaluate whether we could extend the life-span of mice lacking hamartin in neural cells in the brain, specifically in the ependymal lining of the ventricles which give rise to subependymal nodules, through vector-mediated gene replacement.

Further, we evaluated whether this gene therapy is effective when the vector is delivered directly into the circulation in postnatal animals, with the latter being less invasive and having the potential to also alleviate peripheral tissue abnormalities in TSC mouse models and patients. We evaluated this therapeutic strategy with respect to neuropathological features in the brains, especially with respect to hydrocephalus and integrity of the ventricular lining.

4. KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research. Project milestones, such as simply completing proposed experiments, are not acceptable as key research accomplishments. Key research accomplishments are those that have contributed to the major goals and objectives and that have potential impact on the research field.

As part of this funding we were able to complete our development and characterization of a new TSC1 mouse model, published as "Survival benefit and phenotypic improvement by TSC1 gene therapy in a tuberous sclerosis mouse brain model", Prabhakar et al. (2015). In this model, we examined the potential benefit of gene therapy in a mouse model of tuberous sclerosis complex (TSC) in which there is embryonic loss of Tsc1 (hamartin) in all brain neurons. An adeno-associated virus (AAV) vector (serotype rh8) expressing a tagged form of hamartin was injected into the cerebral ventricles of newborn pups with the genotype Tsc1cc (homozygous for a conditional floxed Tsc1 allele) SynI-cre+, in which Tsc1 is lost selectively in neurons starting at embryonic day 12. Vector-treated Tsc1ccSynIcre+ mice showed a marked improvement in survival from a mean of 22 days in non-injected mice to 52 days in AAV hamartin vector-injected mice, with improved weight gain and motor behavior in the latter. Pathologic studies showed normalization of neuron size and a decrease in markers of mTOR activation in the brain in treated as compared to untreated mutant littermates. Hence, we show that gene replacement in the brain is an effective therapeutic approach in this mouse model of TSC1. Our strategy for gene therapy has the advantages that therapy can be achieved from a single application, as compared to repeated treatment with drugs, and that AAV vectors have been found to have minimal to no toxicity in clinical trials for other neurologic conditions. Although there are many additional issues to be addressed, our studies support gene therapy as a useful approach in TSC patients.

Our goal in continuing studies has been to use a mouse model in which loss of hamartin is random in a subset of neurons and other cells throughout the brain starting at P0 (Prabhakar et al., 2013). In this stochastic model, which is more similar to what occurs in patients, we observed subependymal nodules leading to hydrocephalus and death (mean survival of about 60 days). This model requires a two step procedure. First, an intraventricular injection of AAV1-CBA-Cre into the brains of newborn $Tsc1^{c/c}$ pups, with time allowed for formation of subependymal nodules (21 days), but prior to morbidity. Second, at this time point young mice are injected with an AAV-hamartin vector for gene replacement into the vasculature in an attempt to block further growth and formation of subependymal nodules and hence extend the lifespan of the mice.

In the initial phases of this funded period we were compromised as the animals were not breeding prolifically and we had to expand the size of the colony by waiting for the younger mice to reach breeding age. We were told by veterinary experts in our facility that intracerebral ventricular injections (Aim 1) and intravascular tail vein injections (Aim 2) in day 21 old mice would not be feasible as the ketamine (anesthesia used) for the intracerebral ventricular injections can be fatal to such very young animals, and that the tail vein is not accessible for intravascular injection at such an early age due to its small size. Based on this advice we changed our method of delivery of the AAV-hamartin vector to only the retro-orbital vasculature behind the eye which is very safe for mice at any age. Thus, we started our experiments with Aim 2 using the retro-orbital injections into day 21 mice. For these vascular injections we used the AAV-rh8 serotype vector, instead of AAV9 serotype, as the former has also proven very efficient in intravascular delivery of AAV to the brain in mice (Yang et al., 2014).

Aim 1. Evaluate whether intracerebral ventricular injection of AAV1-CBA-hamartin can extend lifespan and reverse neuropathologic abnormalities in a mouse model of brain lesions in Tsc1.

Based on the advice of our veterinarian we have not tried this approach as the young animals do not fare well under the anesthesia used in this procedure. Moreover, we believe that intravascular injection of the vector, as proposed in Aim 2 is more compatible with eventual clinical trials in humans. Given the success of our studies in Aim 2 we are considering a more global model of Tsc1 in mice to see if intravascular gene replacement can also provide therapeutic benefit to other affected organs throughout the body.

Aim 2. Evaluate whether intravascular injection of AAV9-CBA-harmatin can extend lifespan and reverse neuropathologic abnormalities in a mouse model of brain lesions in Tsc1.

We have injected several litters of P0 *Tsc1*$^{c/c}$ pups of mice at P0 with AAV1-CBA-Cre vector [10^{10} (genome copy) g.c. in 2 μl] into each of two ventricles of the brain as proposed in our application (**Fig. 1**). We waited for 21 days, then 50% of the pups were injected with AAVrh8-hamartin and 50% received AAV-rh8-GFP (control vector) by retro-orbital injections in a total volume of 70 μl in one eye (10 μl of 10^{10} g.c. + 60 μl saline; N = 8 pups per group) (**Fig. 2**). We followed the health and survival of these animals at daily intervals. We noted that the AAV-GFP injected animals had several phenotypic abnormalities, including severe hydrocephalus, lethargy, emaciation, hunched posture, etc. as compared to the AAV-hamartin injected animals which appear normal without any of the phenotypic abnormalities mentioned above during the whole survival time.

TSC1$^{c/c}$ pups were injected into both cerebral ventricles with the AAV1-CBA-Cre vector at P0 (day of birth). This procedure has previously been shown to produce subependymal nodules and death by hydrocephalus by around P60 (**Prabhakar et al., 2013**). At P21 days, 12 mice were injected with AAV-rh8-hamartin,12 mice with AAV-rh8-GFP (control) into the retro-orbital vein in the eye (left eye), with 5 mice only injected at P0 with the AAV1-CBA-Cre vector and no retro-orbital injections at P21. Survival curve based on the Log-rank (Mantel-Cox) Test with a statistical significance between hamartin-injected and other groups of $p<0.0001$.

Fig. 1 Intracerebral ventricular injection of AAV1-Cre vector into P0 pups.

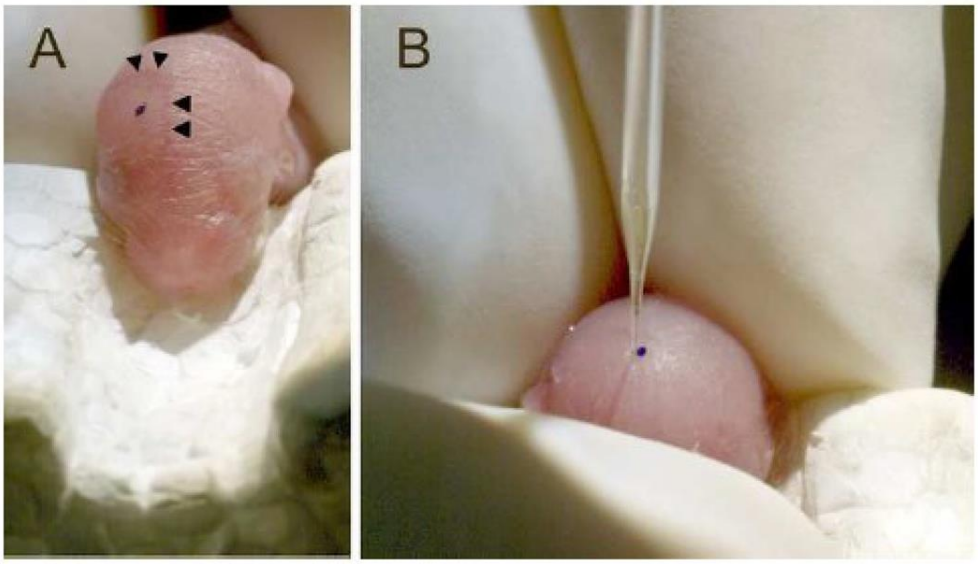
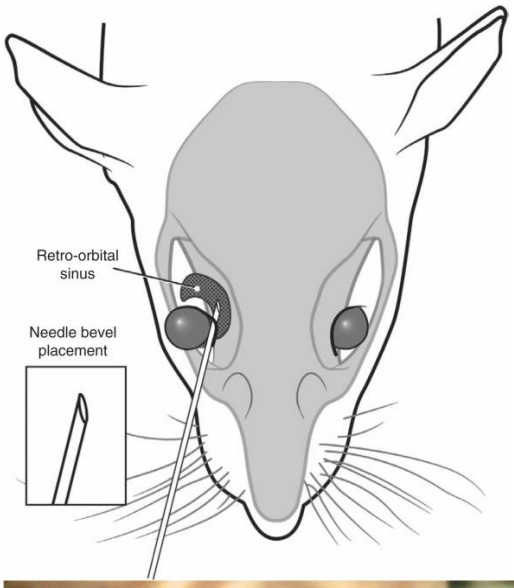


Fig. 2. Retro orbital injection of AAVrh8-TSC1 (hamartin) vector into 3 week old mice.



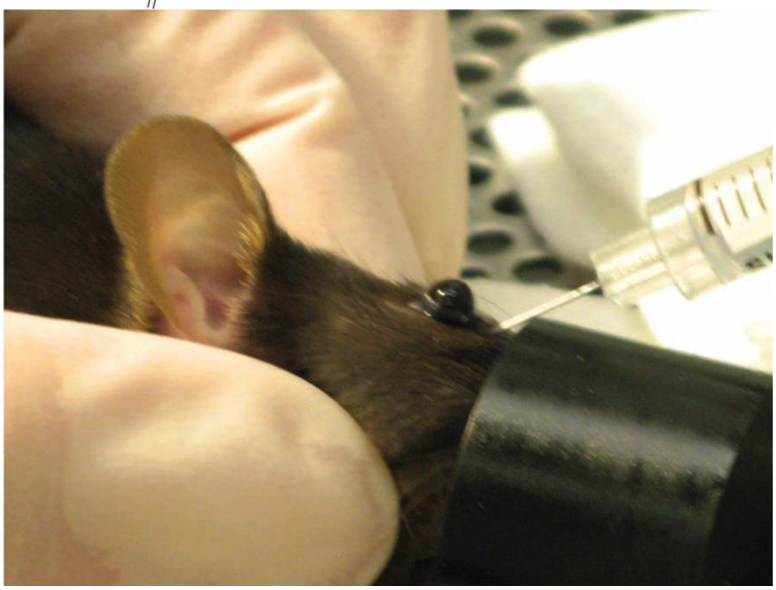
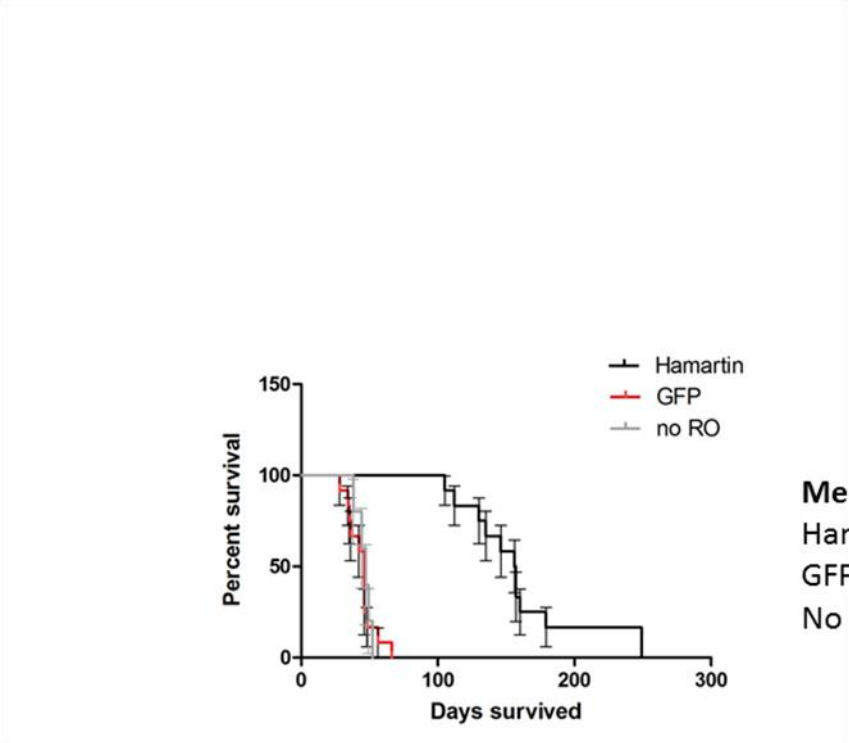


Fig. 3 Gene replacement is able to increase survival of TSC1$^{c/c}$ mice injected with AAV-CBA-Cre vectors.



5. **CONCLUSION:** Summarize the importance and/or implications with respect to medical and /or military significance of the completed research including distinctive contributions, innovations, or changes in practice or behavior that has come about as a result of the project. A brief description of future plans to accomplish the goals and objectives shall also be included.

A parallel set of animals has been sacrificed at periodic intervals, as proposed for histology and immunohistochemistry to evaluate brain neuropathology, specifically evaluating the presence of subependymal nodules. Bouin's fixative has been used to fix the whole animal for Hematoxylin and Eosin (H&E) histology of 2-3 brains from each experimental subgroup with analyses carried out in the HMS Rodent Pathology Core by Dr. Rod Bronson. These analyses include a full body evaluation of tissue abnormalities, as well as assessment of enlarged ventricles. For immunohistochemistry, the brains were fixed fresh in the 2-methyl butane/dry ice bath, sectioned (serial coronal 10 µm) and adjacent sections stained using antibodies for c-Myc, NeuN, GFAP and pS6, or stained for *lacZ* using X-gal. Neuropathological evaluations are being carried out in consultation with Drs. Rod Bronson, Anat Stemmer-Rachamimov and David Kwiatkowski. We will also do immunohistochemical (pS6 staining) and H&E assessment of peripheral tissues, including liver, skeletal muscle, lung and kidney for hamartin-myc expression and any signs of tissue abnormalities (ongoing studies).

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

(1) Lay Press:
(2) Peer-Reviewed Scientific Journals:
(3) Invited Articles:
(4) Abstracts:

Publications:

Prabhakar S, Goto J, Zhang X, Sena-Esteves M, Bronson R, Brockmann J, Gianni D, Wojtkiewicz GR, Chen JW, Stemmer-Rachamimov A, Kwiatkowski DJ, Breakefield XO (2013) Stochastic model of Tsc1 lesions in mouse brain. *PLoS One* 8: doi: 10.1371/annotation/1376a1375b1370a1350-1327e1374-1349bcb1382a-9267dd1363af1353

Prabhakar S, Zhang X, Goto J, Han S, Lai C, Bronson R, Sena-Esteves M, Ramesh V, Stemmer-Rachamimov A, Kwiatkowski DJ, Breakefield XO (2015) Survival benefit and phenotypic improvement by hamartin gene therapy in a tuberous sclerosis mouse brain model. *Neurobiology Disease* **82**: 22–31, doi: 10.1016/j.nbd.2015.04.018.

b. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Poster presentation during the 18th annual ASGCT meeting, May 13th – 16th 2015, New Orleans – title "**AAV-mediated gene replacement therapy in mouse model of tuberous sclerosis"**

7. INVENTIONS, PATENTS AND LICENSES:

List all inventions made and patents and licenses applied for and/or issued. Each entry shall include the inventor(s), invention title, patent application number, filing date, patent number if issued, patent issued date, national, or international.

Nothing to report

8. REPORTABLE OUTCOMES:

Provide a list of reportable outcomes that have resulted from this research. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. This list

may include development of prototypes, computer programs and/or software (such as databases and animal models, etc.) or similar products that may be commercialized.

Prabhakar S, Goto J, Zhang X, Sena-Esteves M, Bronson R, Brockmann J, Gianni D, Wojtkiewicz GR, Chen JW, Stemmer-Rachamimov A, Kwiatkowski DJ, Breakefield XO (2013) Stochastic model of Tsc1 lesions in mouse brain. *PLoS One* 8: doi: 10.1371/annotation/1376a1375b1370a1350-1327e1374-1349bcb1382a-9267dd1363af1353

Prabhakar S, Zhang X, Goto J, Han S, Lai C, Bronson R, Sena-Esteves M, Ramesh V, Stemmer-Rachamimov A, Kwiatkowski DJ, Breakefield XO (2015) Survival benefit and phenotypic improvement by hamartin gene therapy in a tuberous sclerosis mouse brain model. *Neurobiology Disease* **82**: 22–31, doi: 10.1016/j.nbd.2015.04.018.

9. OTHER ACHIEVEMENTS: This list may include degrees obtained that are supported by this award, development of cell lines, tissue or serum repositories, funding applied for based on work supported by this award, and employment or research opportunities applied for and/or received based on experience/training supported by this award.

Based on the work supported by this award, we have applied for the funding to Department of the Army, US Army Medical Research and Materiel Command. Application title : Systemic Gene Therapy for Tuberous Sclerosis. Award Mechanism: FY15 Tuberous Sclerosis Complex Research Program. Exploration : Hypothesis Development Award. Log number : TS150045

For each section, 4 through 9, if there is no reportable outcome, state "Nothing to report."

10. REFERENCES: List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.).

Berhouma M (2010) Management of subependymal giant cell tumors in tuberous sclerosis complex: the neurosurgeon's perspective. *World J Pediatr* **6:** 103-110.

Bevan AK, Duque S, Foust KD, Morales PR, Braun L, Schmelzer L, Chan CM, McCrate M, Chicoine LG, Coley BD, Porensky PN, Kolb SJ, Mendell JR, Burghes AHM, Kaspa BK (2011) Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. *Mol Ther* **19:** 1971-1980.

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Prabhakar S, Goto J, Zhang X, Sena-Esteves M, Bronson R, Brockmann J, Gianni D, Wojtkiewicz GR, Chen JW, Stemmer-Rachamimov A, Kwiatkowski DJ, Breakefield XO (2013) Stochastic model of Tsc1 lesions in mouse brain. *PLoS One* **8**: doi: 10.1371/annotation/1376a1375b1370a1350-1327e1374-1349bc-b1382a-9267dd1363af1353.

Prabhakar S, Zhang X, Goto J, Han S, Lai C, Bronson R, Sena-Esteves M, Ramesh V, Stemmer-Rachamimov A, Kwiatkowski DJ, Breakefield XO (2015) Survival benefit and phenotypic improvement by hamartin gene therapy in a tuberous sclerosis mouse brain model. *Neurobiology Disease* **82**: 22–31, doi: 10.1016/j.nbd.2015.04.018.

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Tyburczy MA, Kotulska K, Pokarowski P, Mieczkowski J, Kucharska J, Grajkowska W, Roszkowski M, Jozwiak S, Kaminska B (2010) Novel Proteins Regulated by mTOR in Subependymal Giant Cell Astrocytomas of Patients with Tuberous Sclerosis Complex and New Therapeutic Implications. *Am J Pathol* **176:** 1878-1890.

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Zhang H, Yang B, Mu X, Ahmed SS, Su Q, He R, Wang H, Mueller C, Sena-Esteves M, Brown R, Xu Z, Gao G (2011) Several rAAV vectors efficiently cross the blood-brain barrier and transduce neurons and astrocytes in the neonatal mouse central nervous system. *Mol Ther* **19:** 1440-1448.

Zhou J, Shrikhande G, Xu J, McKay RM, Burns DK, Johnson JE, Parada LF (2011) Tsc1 mutant neural stem/progenitor cells exhibit migration deficits and give rise to subependymal lesions in the lateral ventricle. *Genes Dev* **25:** 1595-1600.

11. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Two articles and one poster attached. Please double click on the articles and the poster to view full.

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PLOS ONE

Stochastic Model of *Tsc1* Lesions in Mouse Brain

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Abstract

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder due to mutations in either TSC1 or TSC2 that affects many organs with hamartomas and tumors. TSC-associated brain lesions include subependymal nodules, subependymal giant cell astrocytomas and tubers. Neurologic manifestations in TSC comprise a high frequency of mental retardation and developmental disorders including autism, as well as epilepsy. Here, we describe a new mouse model of TSC brain lesions in which complete loss of *Tsc1* is achieved in multiple brain cell types in a stochastic pattern. Injection of an adeno-associated virus vector encoding Cre recombinase into the cerebral ventricles of mice homozygous for a *Tsc1* conditional allele on the day of birth led to reduced survival, and pathologic findings of enlarged neurons, cortical heterotopias, subependymal nodules, and hydrocephalus. The severity of clinical and pathologic findings as well as survival was shown to be dependent upon the dose and serotype of Cre virus injected. Although several other models of TSC brain disease exist, this model is unique in that the pathology reflects a variety of TSC-associated lesions involving different numbers and types of cells. This model provides a valuable and unique addition for therapeutic assessment.

Citation: Prabhakar S, Goto J, Zuang X, Sena-Esteves M, Bronson R, et al. (2013) Stochastic Model of *Tsc1* Lesions in Mouse Brain. PLoS ONE 8(5): e64224. doi:10.1371/journal.pone.0064224

Editor: Jianming Qiu, University of Kansas Medical Center, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

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☯ These authors contributed equally to this work.

Introduction

Tuberous sclerosis complex (TSC) is a genetic disorder affecting about 1 in 6,000 newborns caused by inactivating mutations in *Tsc1* or *Tsc2*, encoding hamartin and tuberin, respectively [1,2]. Biallelic loss of either gene leads to chronic hyperactivation of mTOR complex 1 (mTORC1), and this appears to be the primary pathogenetic mechanism that leads to development of TSC hamartomas in brain, kidney, skin, heart and lung [3,4]. Focal brain pathologies, including cortical tubers and subependymal nodules (SENs), are seen in the majority (>90%) of TSC patients, and have been detected as early as late fetal gestation [5]. TSC tubers disrupt neuronal laminar architecture, and tuber size and number correlate with the incidence of infantile spasms and epileptic seizures [6], as well as global developmental delay [7]. Most TSC patients develop multiple neurological manifestations including seizures, intellectual deficit, neurobehavioral syndromes including autism and autism spectrum disorder, and sleep disorders [3]. Five to 10% of SENs show progressive enlargement, are then called subependymal giant cell astrocytomas (SEGAs), and can lead to devastating neurologic consequences due to blockage of cerebrospinal fluid (CSF) flow and progressive hydrocephalus.

Although there is clear evidence that loss of a single allele of *Tsc1* or *Tsc2* can affect global brain function [8,9], both tuber giant cells and SEGA cells show evidence of complete loss of the TSC1/TSC2 complex with constitutive activation of mTORC1, augmented protein translation [10], reduced autophagy [11,12], and endoplasmic reticulum (ER) and oxidative stress [13]. In addition, cortical tubers contain much higher levels of inflammatory cytokines than normal brain [14], suggesting an inflammatory contribution to TSC brain pathogenesis in humans.

A number of mouse models of TSC brain disease have been generated using conditional alleles of either *Tsc1* or *Tsc2*, and a variety of Cre recombinase alleles driven by different brain-specific promoters, typically active during embryonic development, and in some cases drug-inducible. Promoters have included those selective for neuroprogenitor cells, neurons and astrocytes (e.g. [9,15–23]). In general widespread recombination in brain cells is seen in these models, inducing several features of TSC, such as epileptic seizures, prenatal onset of giant cell development, abnormal brain development (including heterotopias), decreased myelination, and hydrocephalus and premature death. In these conditional models, hamartin or tuberin loss occurs in essentially all of a specific subtype of brain cells at a particular time in development, in contrast to human patients where it occurs in a

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Survival benefit and phenotypic improvement by hamartin gene therapy in a tuberous sclerosis mouse brain model

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ABSTRACT

We examined the potential benefit of gene therapy in a mouse model of tuberous sclerosis complex (TSC) in which there is embryonic loss of Tsc1 (hamartin) in brain neurons. An adeno-associated virus (AAV) vector (serotype rh8) expressing a tagged form of hamartin was injected into the cerebral ventricles of newborn pups with the genotype $Tsc1^{tm}$ (homozygous for a conditional floxed Tsc1 allele) Synl-cre^{+}, in which Tsc1 is lost selectively in neurons starting at embryonic day 12. Vector-treated $Tsc1^{tm}$*Synlcre*$^{+}$ mice showed a marked improvement in survival from a mean of 22 days in non-injected mice to 52 days in AAV hamartin vector-injected mice, with improved weight gain and motor behavior in the latter. Pathologic studies showed normalization of neuron size and a decrease in markers of mTOR activation in treated as compared to untreated mutant littermates. Hence, we show that gene replacement in the brain is an effective therapeutic approach in this mouse model of TSC1. Our strategy for gene therapy has the advantages that therapy can be achieved from a single application, as compared to repeated treatment with drugs, and that AAV vectors have been found to have minimal to no toxicity in clinical trials for other neurologic conditions. Although there are many additional issues to be addressed, our studies support gene therapy as a useful approach in TSC patients.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant disease caused by mutations in *TSC1* or *TSC2*, genes which encode hamartin and tuberin, respectively (Crino, 2013; Kwiatkowski et al., 2010). These proteins are critical in modulating the activity of mTOR which, in turn, regulates development and growth of many tissues (Laplante and Sabatini, 2012). Benign tumors develop in the heart, brain, kidneys, skin, and lungs in TSC patients, and typically follow the classic Knudsen model in which there is a subsequent mutation in the corresponding normal allele ("second hit") occurring in somatic cells resulting in complete loss of either TSC1 or TSC2 expression in cells throughout the body. Neurologic symptoms are seen in over 90% of TSC patients, and include epilepsy, autism spectrum disorders, intellectual disability, attention deficit-hyperactivity, anxiety and sleep disorders (Jülich and Sahin, 2014). Central nervous system (CNS) pathology in TSC includes cortical tubers (focal cortical lesions with giant cells), disorganized architecture with loss of layers in cortical migration tracts, enlarged neurons, reduced myelination and impaired neuronal connectivity (Crino,

Abbreviations: AAV, adeno-associated virus; CBA, chicken beta actin; CMV, cytomegalovirus; CNS, central nervous system; CSF, cerebral spinal fluid; g.c., genome copies; GFAP, glial fibrillary acidic protein; HA-S6K, HA-S6 kinase; H&E, hematoxylin and eosin; HEK, human embryonic kidney; HA, human influenza hemagglutinin; HRP, horseradish peroxide; IHC, immunohistochemistry; IP, immunoprecipitation; i.v., intravenous; IV, intravascular; ICV, intracerebral ventricular; Neu N, neuronal nuclei; pS6, phospho-S6; RIPA, radio immunoprecipitation assay; SEGAs, subependymal giant cell astrocytomas; SMN, spinal motor atrophy; TSC, tuberous sclerosis complex.

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